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AlphaFold 3 sheds insights into chemical enhancer-induced structural changes in Cas12a RNPs

Lulu Pan[1,2*], Aifeng Wang[1], Rui Sang[2], Xia Lu[3,4,5], Wenjie Chen[6], Yongcheng Ma[1*] and Fei Deng[2]

Abstract

Background The CRISPR/Cas12a system has revolutionized nucleic acid detection through trigger-activated nonspecific *trans*-cleavage activity. Enhancing this activity is vital for improving the sensitivity of CRISPR/Cas biosensing systems. Although various chemical enhancers have been shown to affect Cas12a ribonucleoprotein (RNP), the underlying mechanisms remain poorly understood. Investigating how these enhancers alter the structure of Cas12a RNPs is essential for elucidating the enhancement mechanisms involved.

Results This study focuses on elucidating the structural changes in Cas12a RNPs induced by various chemical enhancers via AlphaFold 3, an emerging and powerful tool for analyzing structural changes in protein–nucleic acid complexes. We validated the ability of AlphaFold 3 to simulate structural changes in Cas12a RNPs activated by triggers and subsequently analyzed the effects of specific enhancers, such as reducing agents (e.g., Dithiothreitol, namely DTT), divalent cations (e.g., Mg^{2+}, Mn^{2+}), and bovine serum albumin (BSA). Our findings revealed that DTT, simulated with a cysteine-to-serine Cas12a mutant, caused significant structural changes in Cas12a RNP, as evidenced by notable shifts in the distance between key residues (Val377 to Gln1136) and a high root mean square deviation (RMSD) (RMSD > 2). Conversely, divalent cations and BSA did not cause substantial structural changes, resulting in only minor shifts in residue distance and a low RMSD (RMSD < 2).

Conclusions These results demonstrate that DTT enhances Cas12a activity by inducing significant structural rearrangements, whereas divalent cations and BSA-induced enhancements do not involve substantial structural modifications.

Keywords CRISPR/Cas12a, AlphaFold 3, Chemical enhancers, Ribonucleoprotein (RNP), Structural simulation

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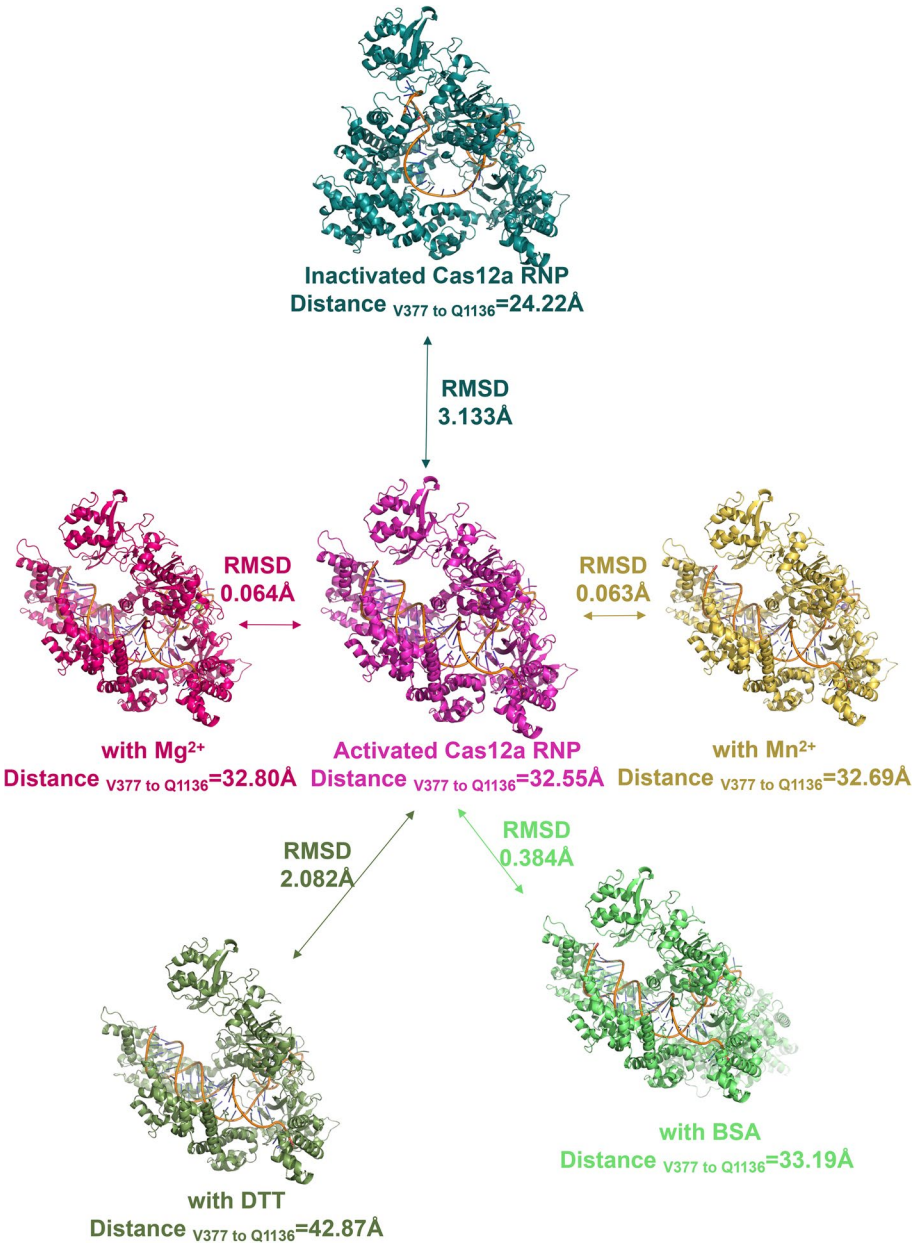
Graphical Abstract

Summary of structural changes from inactivated Cas12a RNP to activated Cas12a RNP and from activated Cas12a RNP to activated Cas12a RNP with an enhancer. The units of both the RMSD and distance are Å.



Background

The CRISPR/Cas system, which was originally discovered as a defense mechanism in bacteria and archaea against viral infections, comprises CRISPR sequences and Cas proteins [1]. This technology has revolutionized biomedical and biotechnological applications, particularly nucleic acid detection, owing to its efficiency and specificity, with systems such as DETECTR (DNA endonuclease-targeted CRSIPR trans reporter) [2], HOLMES (one-hour low-cost multipurpose highly efficient system) [3], and SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) [4]. DETECTR and HOLMES utilize CRISPR/

Cas12a, which employs nonspecific *trans*-cleavage mediated by crRNA to amplify signals upon target recognition [5]. The structure of Cas12a includes RuvC and NUC (nuclease) domains; the former is responsible for target recognition and the specific cleavage of both the target and nontarget strands, whereas the latter guides strands to the active site, making the Cas12a ribonucleoprotein (RNP) pivotal in nucleic acid detection [6].

Increasing the *trans*-cleavage activity of Cas nucleases, which is crucial for the sensitivity of CRISPR/Cas biosensing systems, can be achieved through various chemical methods, such as the use of enhancers. Common enhancers of Cas12a include reducing agents (e.g., DTT) [7, 8], divalent cations (e.g., Mg^{2+}, Mn^{2+}) [9–11], and BSA [12]. While DTT has been found to increase the activity of the RNP of the Lachnospiraceae bacterium Cas12a (LbCas12a) by causing conformational changes [7], the current structural evidence is insufficient. Additionally, comprehensive structural studies on other enhancers are lacking. For example, the structural influence of divalent cations and BSA on Cas12a RNPs remains unclear, despite evidence suggesting that divalent cations can stimulate pre-crRNA processing of FnCas12a RNPs [11] and stabilize the conformation of crRNA of LbCas12a RNPs [10].

X-ray crystallography and cryo-electron microscopy (cryo-EM) have been successfully used in structural studies of the CRISPR/Cas system [6, 13–15]. However, these methods have limitations, such as difficulty in obtaining high-quality crystals of large or flexible molecules via X-ray crystallography and the high cost and technical challenges associated with Cryo-EM. Computational models are therefore important for solving protein folding problems. AlphaFold, a neural network approach, uses deep learning to accurately predict the three-dimensional structure of proteins, including 98.5% of human protein structures [16]. AlphaFold 3, the first AI system to go beyond real biomolecular structure prediction tools, can accurately predict complex structures involving proteins, nucleic acids, and small molecules [17]. This capability offers a unique opportunity to investigate how enzyme enhancers influence Cas12a RNP structures, facilitating a deeper understanding of the mechanisms through which these enhancers increase CRISPR/Cas system sensitivity.

This study focuses on elucidating structural changes in Cas12a RNPs induced by enhancers via AlphaFold 3. Initially, AlphaFold 3 was validated for its ability to simulate structural changes in Cas12a RNPs activated by triggers. The effects of specific enhancers on Cas12a RNP structures were subsequently analyzed. Our results demonstrated that DTT-induced enhancement, simulated via a cysteine-to-serine Cas12a mutant, significantly altered the structure compared with activated Cas12a RNP states, whereas other enhancers, such as divalent cations and BSA, did not induce detectable structural changes. This work provides structural insights into how commonly used enhancers affect Cas12a RNP activity, leveraging AlphaFold 3 to deepen our understanding of CRISPR/Cas systems in nucleic acid detection.

Methods

Structural simulation of Cas12a RNPs

The AlphaFold Server, powered by AlphaFold 3, was used to simulate the structure of the Cas12a RNP. The sequences are provided in Supplementary Table 1. The protein and/or guide RNA and/or DNA trigger sequence, as well as ions were input into separate column with responding molecular type, the copies of protein, RNA and DNA were set as one, the copies of Mg2 + and Mn2 + were set as two. Then the job was submitted with Auto seed. A pTM (predicted template modeling) score above 0.5 indicates that the overall predicted fold for the complex might be similar to the true structure. ipTM (interface-predicted template modeling) measures the accuracy of the predicted relative positions of the subunits within the complex, with values higher than 0.8 representing confident high-quality predictions [18, 19].

Structural comparison of different complexes

The structures of different complexes were compared via the PyMOL Molecular Graphics System (Version 2.6 Schrödinger, LLC). Protein profiles (model-0.cif) from PDB or AlphaFold 3 were loaded into PyMOL. The structure of one complex was aligned with that of another complex to view the comparable structures and obtain

(See figure on next page.)

Fig. 1 Structural analysis of Cas12a RNPs via PDB and AlphaFold3. **A** Structural comparison between PDB 6NME (inactivated Cas12a RNP, yellow) and 5XUS (activated Cas12a RNP, green) (RMSD = 2.567). **B** Molecular estimates of distance changes between Val377 and Gln1136 due to changes in the Cas12a conformation before and after activation. The Val377–Gln1136 distance in inactivated RNPs is 24.59 Å, and in activated RNPs, it is 30.13 Å. Green: Cas12a protein; blue: nuclease domain (NUC) in Cas12a; brown: gRNA; slate: ssDNA trigger. **C** Structural comparison between the simulation model based on AlphaFold3 using the same sequence as PDB 6NME (inactivated Cas12a RNP, purple) and 5XUS (activated Cas12a RNP, cyan) (RMSD = 2.953). **D** Molecular simulation estimates of distance changes between Val377 and Gln1136 due to changes in the Cas12a conformation before and after activation by AlphaFold3. The Val377–Gln1136 distance in inactivated RNPs is 24.22 Å, and in activated RNPs, it is 30.86 Å. Green: Cas12a protein; blue: nuclease domain (NUC) in Cas12a; brown: gRNA; slate: ssDNA trigger

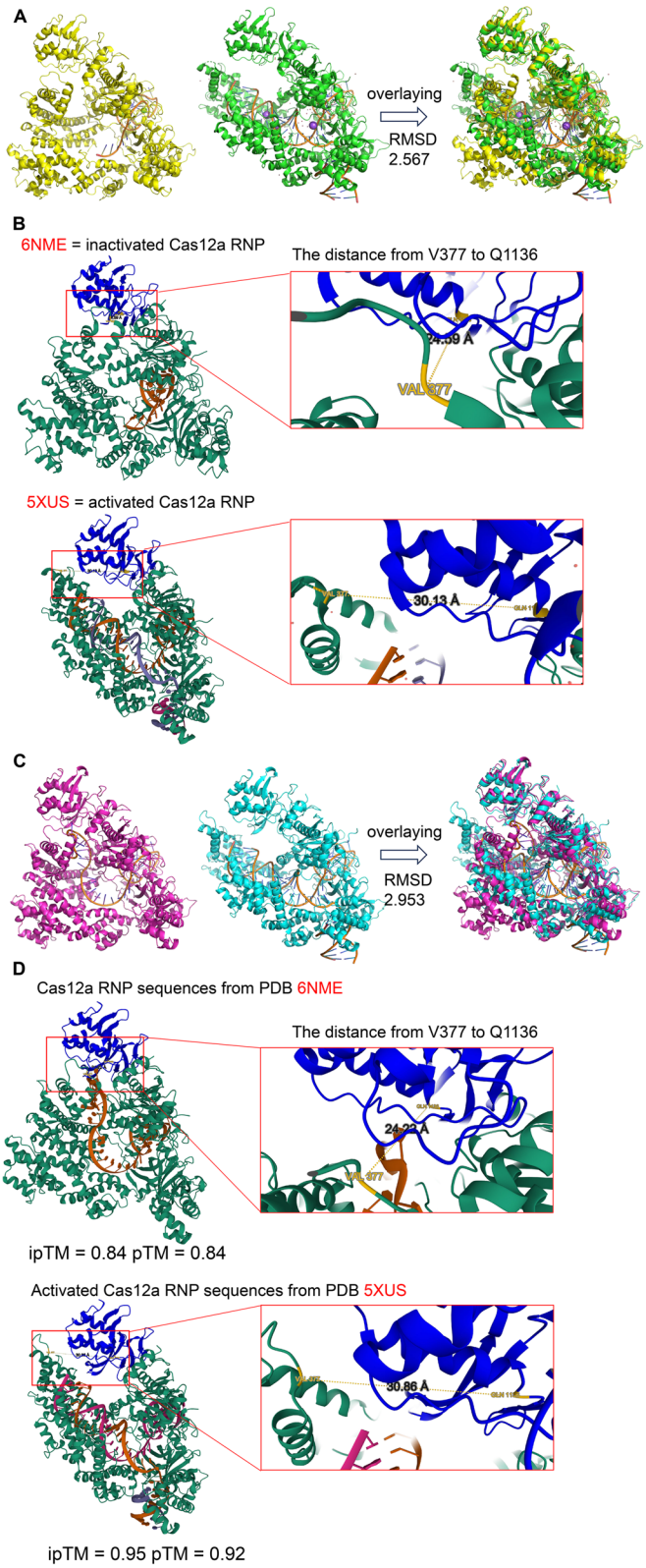


Fig. 1 (See legend on previous page.)

the root mean square deviation (RMSD). An RMSD value greater than 2 Å indicates significant differences between the comparable complexes [20]. PDB 5XUS represents activated Cas12a RNP; PDB 6NME represents inactivated Cas12a RNP.

Measurement of the Val377-to-Gln1136 distance

Distances between Val377 and Gln1136 in Cas12a RNPs were simulated and measured via Visual Studio Code (VS Code, Version 1.91.1). Protein profiles from PDB or AlphaFold 3 were loaded into VS Code, the protein viewer of model-0.cif was launched, and the distance measurement tool was selected. Finally, the distance between the corresponding residues was measured on the 3D structure model.

Prediction of intramolecular disulfide bonds

Intramolecular disulfide bonds were predicted via Design v2.13 [21] (http://cptweb.cpt.wayne.edu/DbD2/index.php). LbCas12a (.pdb) was loaded into Design v2.13, and RUN was pressed to find disulfide bonds via default parameters.

Results

AlphaFold3 simulates structural changes in Cas12a RNPs when activated by triggers

To analyze the global conformational changes in Cas12a RNPs before and after activation, the structures of inactivated (6NME) [15] and activated (5XUS) [6] Cas12a RNPs from PDB were compared via PyMOL, which revealed a significant difference, with an RMSD of 2.567 Å (Fig. 1A). Because the NUC domain is an important region of Cas12a for cleavage activity, the conformation around NUC could be more important than other regions. The distance changes between Val377 and Gln1136 in Cas12a were chosen to reflect Cas12a activation, as Gln1136 is in the NUC domain of Cas12a and Val377 is in the REC2 region, which is closer to the NUC in the inactivated state and moves away upon activation [6]. The distance between Val377 and Gln1136 was 24.59 Å in the inactivated RNP and 30.13 Å in the activated RNP (Fig. 1B). The same sequences as inactivated (6NME) and activated (5XUS) Cas12a RNP from PDB were then used to assess the accuracy of simulation by AlphaFold3, which displayed similar results as PDB, with an RMSD of 2.953 Å between inactivated and activated Cas12a RNP and Val377 to Gln1136 distances ranging from 24.22 Å in inactivated RNP to 30.13 Å in activated RNP (Fig. 1C-D). Direct structural comparison of the same Cas12a RNP between PDB and AlphaFold3 indicated that AlphaFold3 could simulate the structures of Cas12a RNPs with high accuracy (Supplementary Fig. 1). To assess the versatility of AlphaFold 3 with different triggers, we used an alternate set of gRNA and trigger, which also showed significant structural differences, with an RMSD of 3.133 Å between inactivated and activated Cas12a RNPs (Fig. 2A). The distance between Val377 and Gln1136 increased from 24.22 Å in the inactive RNP to 32.55 Å in the activated RNP (Fig. 2B). These results confirmed that AlphaFold 3 effectively simulates structural changes in Cas12a RNPs upon activation with high accuracy and versatility.

DTT-enhanced activity of Cas12a RNPs could be simulated by cysteine to serine mutation on the basis of AlphaFold3

Dithiothreitol (DTT) is a commonly used reducing agent to break unnatural disulfide bridges, potentially changing the conformation of the Cas12a RNP and enhancing its activity. However, the structural evidence was still insufficient, and direct simulation of Cas12a RNP with DTT was not feasible with AlphaFold 3 since DTT cannot be added into the structural model via AlphaFold 3. Instead, we simulated the effect by mutating all cysteine residues to serine residues, which share similar characteristics except for the formation of disulfide bonds, and the mutation did not influence the structure of Cas12a itself or inactivate Cas12a RNP (Supplementary Figs. 3–4). Structural comparisons between simulation models of the activated Cas12a RNP and activated mutCas12a (cysteine to serine Cas12a mutant) RNP revealed differences, with an RMSD of 2.082 Å (Fig. 2C), and the distances between

(See figure on next page.)

Fig. 2 Structural analysis of inactivated, activated and activated cysteine-to-serine Cas12a mutant RNPs on the basis of AlphaFold3. **A** Structural comparison between the simulation model based on AlphaFold3 via inactivated Cas12a RNP (deepteal) and activated Cas12a RNP (purple) (RMSD = 3.133). **B** Molecular simulation estimates of distance changes between Val377 and Gln1136 due to changes in the Cas12a conformation before and after activation by AlphaFold3. The Val377–Gln1136 distance in inactivated RNPs is 24.22 Å, and in activated RNPs, it is 32.55 Å. Green: Cas12a protein; blue: nuclease domain (NUC) in Cas12a; brown: gRNA; slate: ssDNA trigger. **C** Structural comparison between the simulation model based on AlphaFold3 using the activated Cas12a RNP (purple) and the cysteine-to-serine Cas12a mutant RNP (smudge) (RMSD = 2.082). **D** Molecular simulation estimates of distance changes between Val377 and Gln1136 due to the Cas12a conformation changes resulting from cysteine to serine mutations by AlphaFold3. The Val377–Gln1136 distance from the activated cysteine-to-serine Cas12a mutant RNP is 42.87 Å, which is greater than the 32.55 Å distance in the activated Cas12a RNP. Green: Cas12a protein; blue: nuclease domain (NUC) in Cas12a; brown: gRNA; slate: ssDNA trigger

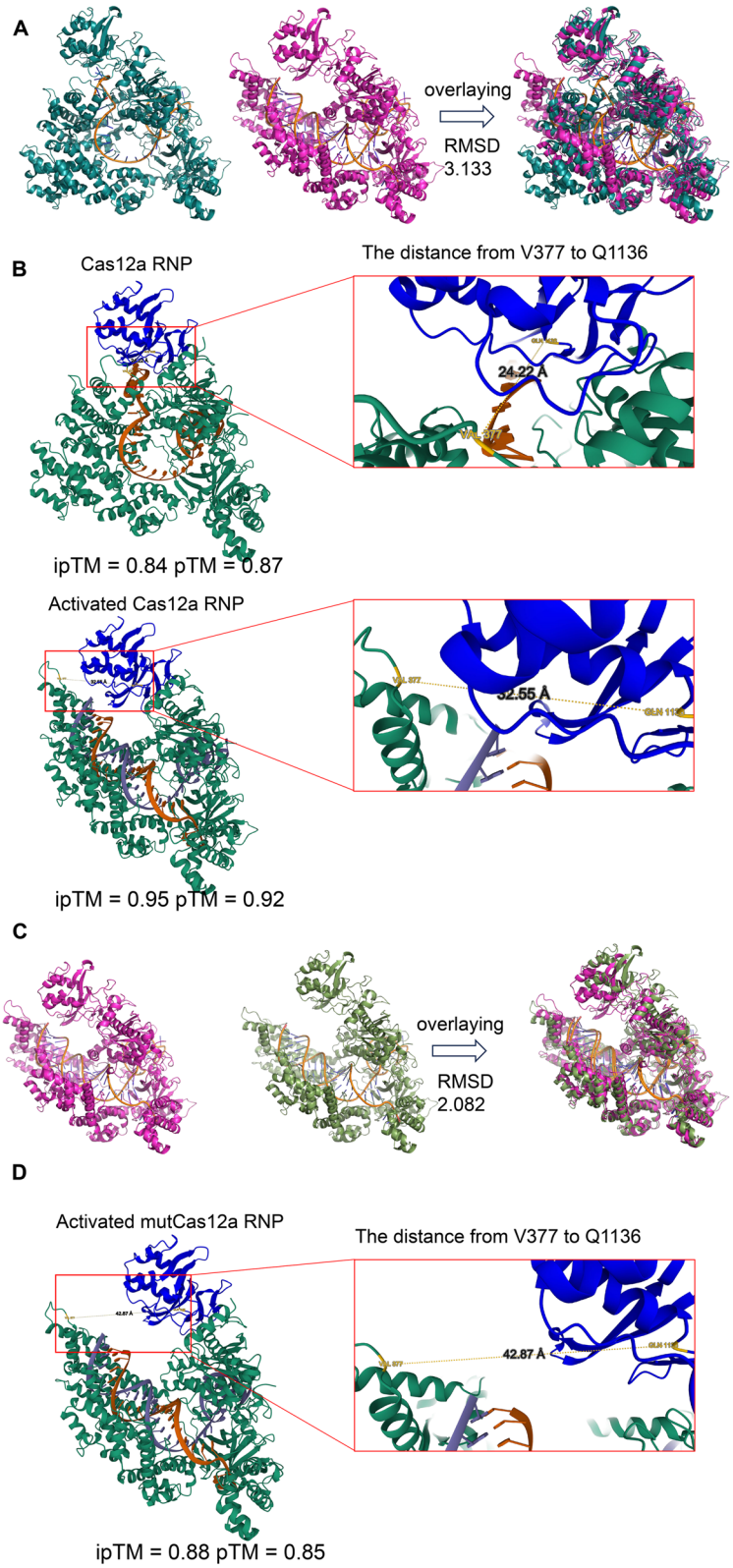


Fig. 2 (See legend on previous page.)

Val377 and Gln1136 changed from 32.55 Å in the activated Cas12a RNP to 42.87 Å in the activated mutCas12a RNP (Fig. 2D). These results indicated that DTT-induced enhancement, simulated via mutCas12a, significantly altered the structure compared with activated Cas12a RNP states, demonstrating that DTT-induced enhancement can be simulated via cysteine-to-serine mutation via AlphaFold 3.

Divalent cations did not induce significant structural changes in Cas12a RNPs

Divalent cations, such as Mg^{2+} and Mn^{2+}, are known to increase the activity of Cas12a RNPs. To evaluate their impact on the Cas12a RNP structure, we conducted a structural analysis via AlphaFold 3. For Mg^{2+}, two ions were incorporated into the simulated structure on the basis of the PDB data. The structural comparison between the activated Cas12a RNP with Mg^{2+} and the activated Cas12a RNP revealed minimal differences (RMSD = 0.064) (Fig. 3A). The distance between Val377 and Gln1136 in the Mg^{2+}-bound activated Cas12a RNP is 32.80 Å, which is comparable to the 32.55 Å distance observed in the activated Cas12a RNP (Fig. 3B). Similarly, for Mn^{2+}, two ions were added to the simulation, which also revealed no significant structural changes compared with the activated Cas12a RNP (RMSD = 0.063) (Fig. 3C). The distance between Val377 and Gln1136 was 32.69 Å in the Mn^{2+}-bound structure, close to the 32.55 Å distance observed in the activated Cas12a RNP (Fig. 3D). These findings indicated that divalent cations do not induce structural changes in Cas12a RNPs, and AlphaFold 3 simulations cannot capture the enhanced activity of Cas12a RNPs because of these divalent cations.

BSA does not induce significant structural changes in Cas12a RNPs

In addition to DTT and divalent cations, BSA has been reported as another chemical enhancer for Cas12a RNPs. To test whether BSA could induce structural changes in Cas12a, we simulated activated Cas12a RNP with BSA via AlphaFold 3. The structure of activated Cas12a RNP with BSA was not different from that of activated Cas12a RNP without BSA, with an RMSD of 0.384 (Fig. 4A) and a distance between Val377 and Gln1136 of 33.19 Å, comparable to the 32.55 Å in the activated Cas12a RNP (Fig. 4B). These results indicated that BSA could not induce structural changes in Cas12a RNPs, suggesting that AlphaFold 3 simulations may not accurately reflect the BSA-enhanced activity of Cas12a RNPs.

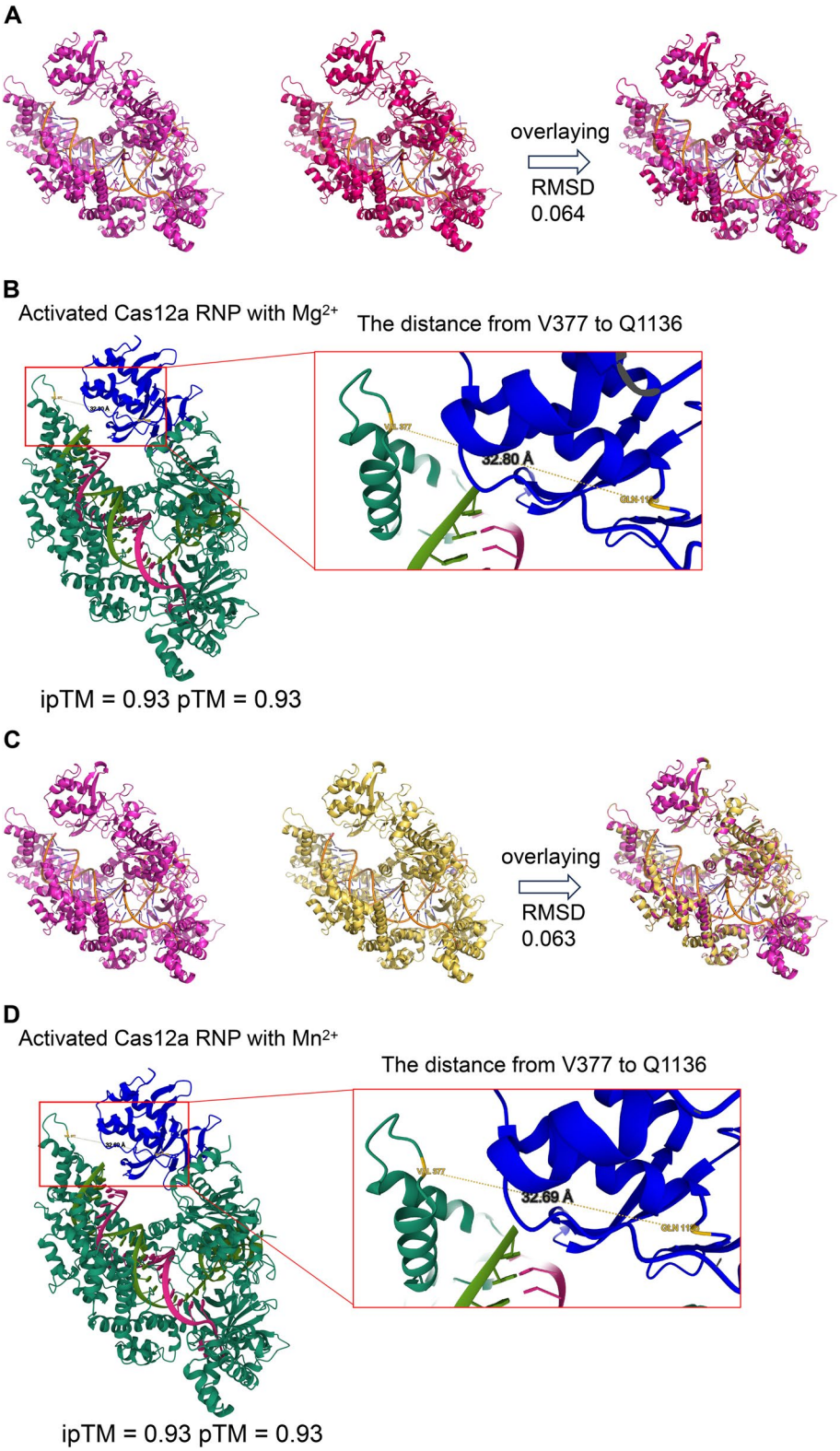
Discussion

The RMSD is a common metric used to compare the three-dimensional coordinates of protein or macromolecular structures [20, 22]. Typically, an RMSD value less than 2 Å between two protein structures indicates high structural similarity, whereas a higher RMSD value may suggest significant structural changes. Direct structural comparison of the same Cas12a RNP between PDB and AlphaFold3 found that the RMSD was less than 2 Å, indicating that AlphaFold3 could simulate the structures of Cas12a RNPs with high accuracy (Supplementary Fig. 1).

Given that the NUC domain is crucial for Cas12a cleavage activity, we focused on the distance between Val377 and Gln1136 as a marker of Cas12a activation. Gln1136 is located within the NUC domain, whereas Val377 is situated in the REC2 region, which is closer to the NUC in the inactivated state and moves away upon activation [6]. The measurements of Val377 to Gln1136 distances and RMSD (Supplementary Table 2–3), especially for the structural changes from inactivated Cas12a RNP to activated Cas12a RNP and from activated Cas12a RNP to activated Cas12a RNP with an enhancer, confirmed that changes in the distance between Val377 and Gln1136 could provide supplementary evidence for Cas12a activation and conformational changes in conjunction with RMSD analysis. Meanwhile, the similarity of Val377 to Gln1136 distances from the same Cas12a RNP between PDB and AlphaFold3 confirmed the reliability of AlphaFold to simulate the structures of Cas12a RNPs, providing idea for chemical enhancer-induced structural changes in Cas12a RNPs.

(See figure on next page.)

Fig. 3 Structural analysis of activated Cas12a RNPs with divalent cations on the basis of AlphaFold3. **A** Structural comparison between the simulation model based on AlphaFold3 using the activated Cas12a RNP (purple) and the activated Cas12a RNP with Mg^{2+} (red) (RMSD = 0.064). **B** Molecular simulation estimates of distance changes between Val377 and Gln1136 due to the Cas12a conformation changes resulting from Mg^{2+} by AlphaFold3. The Val377–Gln1136 distance in activated Cas12a RNPs with Mg^{2+} is 32.80 Å, which is similar to the 32.55 Å distance in activated Cas12a RNPs. Green: Cas12a protein; blue: nuclease domain (NUC) in Cas12a; parakeet: gRNA; magenta: ssDNA trigger; pink: Mg^{2+}. **C** Structural comparison between the simulation model based on AlphaFold3 using the actual activated Cas12a RNP (purple) and activated Cas12a RNP with Mn^{2+} (yellow) (RMSD = 0.063). **D** Molecular simulation estimates of distance changes between Val377 and Gln1136 due to the Cas12a conformation changes resulting from Mn^{2+} by AlphaFold3. The Val377–Gln1136 distance in activated Cas12a RNPs with Mn^{2+} is 32.69 Å, which is similar to the 32.55 Å distance in activated Cas12a RNPs. Green: Cas12a protein; blue: nuclease domain (NUC) in Cas12a; parakeet: gRNA; magenta: ssDNA trigger; pink: Mn^{2+}



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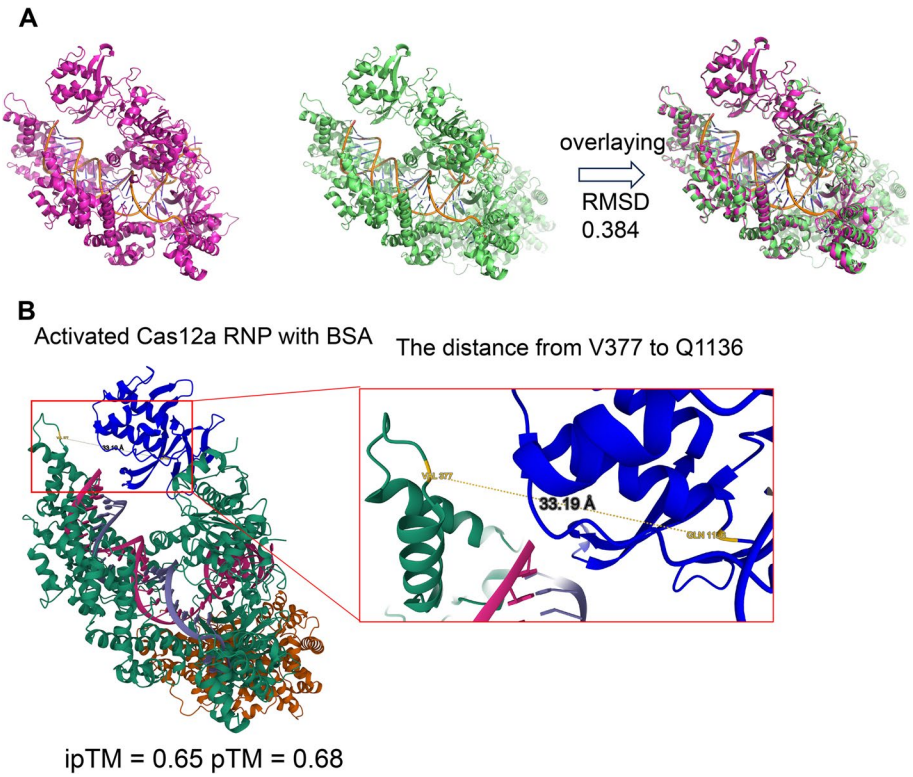

Fig. 4 Structural analysis of activated Cas12a RNP with BSA on the basis of AlphaFold3. **A** Structural comparison between simulation models based on AlphaFold3 via the activated Cas12a RNP (purple) and the BSA-enhanced activated Cas12a RNP (palegreen) (RMSD = 0.384). **B** Molecular simulation estimates of distance changes between Val377 and Gln1136 due to the Cas12a conformation changes resulting from BSA by AlphaFold3. The Val377–Gln1136 distance in activated Cas12a RNP with BSA is 33.19 Å, which is similar to the 32.55 Å distance in activated Cas12a RNP. Green: Cas12a protein; blue: nuclease domain (NUC) in Cas12a; parakeet: gRNA; slate: ssDNA trigger; brown: BSA

Direct simulation of the Cas12a RNP with DTT was not feasible, as DTT cannot be input into AlphaFold3. Recent research has shown that mutating multiple cysteine residues to serine residues can increase the activity of AsCas12a in cells [23]. LbCas12a contains nine cysteines (Supplementary Fig. 2), none of which are involved in intramolecular disulfide bonds according to Design v2.13 (http://cptweb.cpt.wayne.edu/DbD2/index.php). Thus, substituting all cysteine residues with serine residues, which share similar properties except for disulfide bond formation, can simulate the effect of DTT without altering the intrinsic structure of Cas12a or its inactive RNP form (Supplementary Figs. 3–4). Our results indicated that the structural changes induced by DTT, simulated via mutCas12a, significantly affected the structure compared with the activated Cas12a RNP state, demonstrating that DTT-enhanced Cas12a RNP activity can be effectively modeled via cysteine-to-serine mutations in AlphaFold3. Moreover, mutCas12a could be utilized in experimental settings to increase Cas nuclease trans-cleavage activity, even in the absence of DTT or other reducing agents.

Our simulation of divalent cation-bound Cas12a RNPs suggested that divalent cations such as Mg^{2+} and Mn^{2+} might act as enhancers by stabilizing crRNA conformation rather than inducing structural changes in LbCas12a RNPs. However, AlphaFold3 could not verify interactions involving $(Mg(H_2O)_6)^{2+}$ and crRNA, which was reported previously [10], because of the inability to input H_2O into the simulation model. Research has shown that only Mn^{2+} can activate the crRNA-independent DNase activity of LbCas12a, whereas Mg^{2+} does not have this effect [24]. We then simulated the structural change in LbCas12a caused by Mn^{2+} and Mg^{2+} and found that neither Mn^{2+} nor Mg^{2+} caused a significant structural change in LbCas12a; however, the RMSD between Cas12a and Mn^{2+}-bound Cas12a was 1.910 Å, which is close to the significance threshold of 2 Å. These findings suggested that Mn^{2+} partially activated LbCas12a by inducing slight conformational changes (Supplementary Fig. 5). Therefore, we speculated that Mg^{2+} and Mn^{2+} might stabilize the crRNA conformation rather than induce structural changes in

LbCas12a RNPs, whereas Mn^{2+} could affect the structure of LbCas12a in the absence of crRNA.

For the simulation of Cas12a RNP with BSA, the pTM score was 0.68, indicating that the overall predicted fold of the complex may resemble the true structure [18, 19]. The ipTM score ranged between 0.6 and 0.8 (0.65), suggesting variable accuracy in predicting the relative positions of the subunits. Considering that the RMSD reflects overall fold similarity between complexes and pTM scores, the overall predicted folds of the complex for activated Cas12a RNP and BSA-enhanced activated Cas12a RNP were 0.92 (Fig. 2B) and 0.68 (Fig. 4B), respectively, and the RMSD between these two states might be accurate. This finding suggests that BSA enhances Cas12a RNPs not by inducing significant structural changes, but likely through one or more of the following mechanisms: 1) stabilization of Cas proteins and other macromolecules in various reactions; 2) neutralization of unknown inhibitors within the system, particularly those arising from protein purification or nucleic acid preparation; and 3) promotion of the refolding of Cas proteins by modulating solvent properties [12].

Conclusion

In this study, we investigated the structural changes in Cas12a RNPs induced by various chemical enhancers via the computational tool AlphaFold 3. Our findings demonstrated that while AlphaFold 3 can accurately simulate the conformational changes in Cas12a RNPs upon activation, it also effectively models the structural changes induced by certain enhancers. Specifically, we observed significant structural alterations in the presence of DTT, which were simulated via a cysteine-to-serine Cas12a mutation, as evidenced by the substantial change in the distance between the Val377 and the Gln1136 residues and an RMSD value greater than 2 Å, indicative of notable conformational shifts. This suggested that DTT may increase Cas12a activity by inducing significant structural rearrangements. Conversely, the application of divalent cations (Mg^{2+}, Mn^{2+}) and BSA did not result in detectable structural changes, with a small change in the distance between the Val377 residue and the Gln1136 residue and an RMSD remaining below 2 Å. These results implied that the enhancement of Cas12a activity by these agents may not involve substantial structural modifications but could instead be attributed to other factors, such as the stabilization of the crRNA conformation or other mechanistic pathways. The insights gained from this study deepen our understanding of the molecular mechanisms underlying enhancer-induced activity modulation in CRISPR/Cas systems, highlighting the potential and limitations of computational approaches in structural biology.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44301-024-00003-z.

Supplementary Material 1.

Supplementary Material 2.

Authors' contributions

Lulu Pan: Methodology, Data analysis, Writing—original draft, Aifeng Wang: Methodology. Rui Sang: Methodology. Xia Lu: Data analysis. Wenjie Chen: Data analysis. Yongcheng Ma: Project administration, Writing -review & editing. Fei Deng: Supervision, Methodology, Writing -review & editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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References

1. Rodolphe B. The roles of CRISPR-Cas systems in adaptive immunity and beyond. Curr Opin Immunol. 2015;32:36–41.
2. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436–9.
3. Yi Y, Li P, Huang Y, et al. P21-activated kinase 2-mediated beta-catenin signaling promotes cancer stemness and osimertinib resistance in EGFR-mutant non-small-cell lung cancer. Oncogene. 2022;41(37):4318–29.
4. Gootenberg JS, Abudayyeh OO, Lee JW, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438–42.
5. Li S, Cheng Q, Liu J, et al. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. Cell Res. 2018;28(4):491–3.
6. Yamano T, Zetsche B, Ishitani R, et al. Structural Basis for the Canonical and Non-canonical PAM Recognition by CRISPR-Cpf1. Mol Cell. 2017;67(4):633-645.e633.
7. Deng F, Li Y, Li B, et al. Increasing trans-cleavage catalytic efficiency of Cas12a and Cas13a with chemical enhancers: Application to amplified nucleic acid detection. Sens Actuators, B Chem. 2022;373: 132767.
8. Deng F, Li Y, Yang B, et al. Topological barrier to Cas12a activation by circular DNA nanostructure s facilitates autocatalysis and transforms DNA/RNA sensing. Nat Commun. 2024;15(1):1818.

9. Ma P, Meng Q, Sun B, et al. MeCas12a, a Highly Sensitive and Specific System for COVID-19 Detection. Advanced science. 2020;7(20):2001300.
10. Dong D, Ren K, Qiu X, et al. The crystal structure of Cpf1 in complex with CRISPR RNA. Nature. 2016;532(7600):522–6.
11. Swarts DC, van der Oost J, Jinek M. Structural Basis for Guide RNA Processing and Seed-Dependent DNA Targe ting by CRISPR-Cas12a. Mol Cell. 2017;66(2):221-233.e224.
12. Li Z, Zhao W, Ma S, et al. A chemical-enhanced system for CRISPR-Based nucleic acid detection. Biosens Bioelectron. 2021;192: 113493.
13. Takeda SN, Nakagawa R, Sae O, et al. Structure of the miniature type V-F CRISPR-Cas effector enzyme. Mol Cell. 2021;81(3):558-570 e553.
14. Xiao R, Li Z, Wang S, et al. Structural basis for substrate recognition and cleavage by the dimeriz ation-dependent CRISPR-Cas12f nuclease. Nucleic Acids Res. 2021;49(7):4120–8.
15. Zhang H, Li Z, Daczkowski CM, et al. Structural Basis for the Inhibition of CRISPR-Cas12a by Anti-CRISPR Proteins. Cell Host Microbe. 2019;25(6):815-826.e814.
16. Jumper J, Evans R, Pritzel A, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;596(7873):583–9.
17. Abramson J, Adler J, Dunger J, et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature. 2024;630(8016):493–500.
18. Xu J, Zhang Y. How significant is a protein structure similarity with TM-score = 0.5? Bioinformatics. 2010;26(7):889–95.
19. Zhang Y, Skolnick J. Scoring function for automated assessment of protein structure templat equality. Proteins. 2004;57(4):702–10.
20. Mukherjee S, Balius TE, Rizzo RC. Docking validation resources: protein family and ligand flexibility experiments. J Chem Inf Model. 2010;50(11):1986–2000.
21. Craig DB, Dombkowski AA. Disulfide by Design 2.0: a web-based tool for disulfide engineering in proteins. BMC Bioinformatics. 2013;14:346.
22. Meli R, Biggin PC. spyrmsd: symmetry-corrected RMSD calculations in Python. Journal of cheminformatics. 2020;12(1):49.
23. Vakulskas CA, Bode NM, Collingwood MA et al: CAS12a mutant genes and polypeptides encoded by same. In., vol. US 2020/0216825 A1, 9 Jul 2020. US Patent 2020/0216825 A1, 9 Jul 2020; 2020.
24. Li B, Yan J, Zhang Y, et al. CRISPR-Cas12a Possesses Unconventional DNase Activity that Can Be Inactivated by Synthetic Oligonucleotides. Mol Ther Nucleic Acids. 2020;19:1043–52.

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